

# **Preparation and sterilization Of culture media**

## **Culture of bacteria**

### **Streak plate method**

**Done by Anas Zayad**

The media provides the proper environment for growth of bacteria.

**Media** : referring to the substances were organism grown , it design to mimic the environment which the bacteria grown naturally

Sugar

Nitrogen

Elements

pepton

D.W

## Types of media:

### 1. Liquid media (broth):

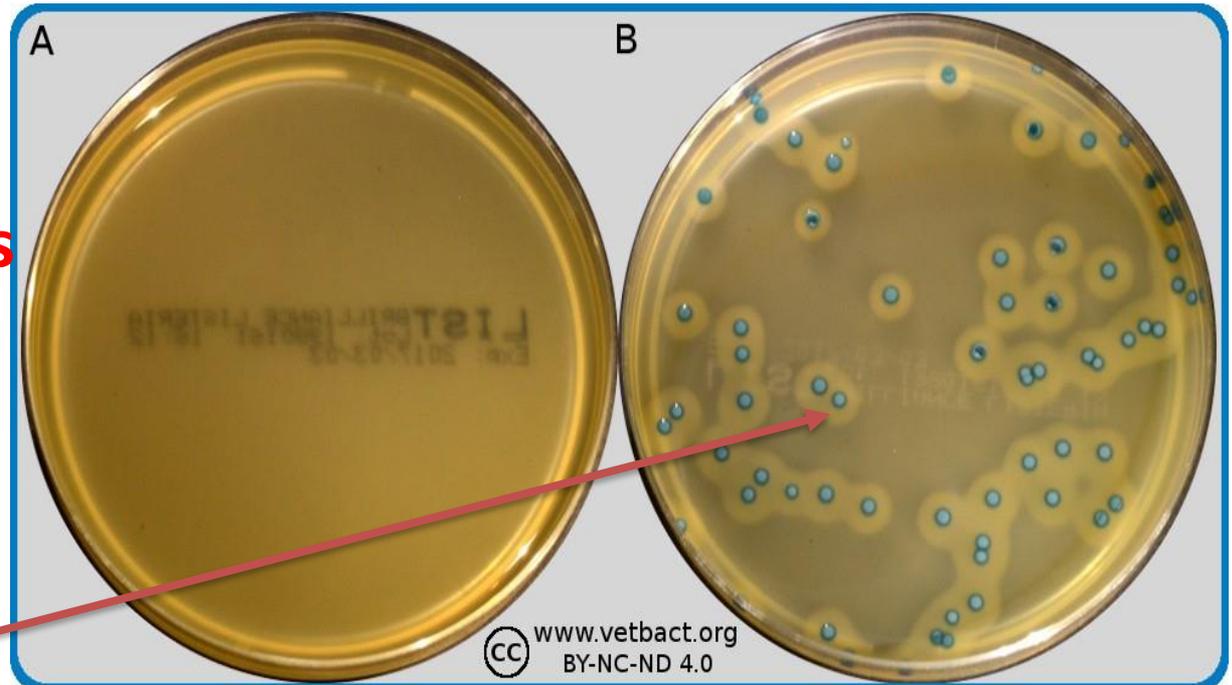
- we put the liquid media inside a tube.
- turbidity (العكرة) is used as an indicator for bacterial growth.

Turbidity increases (more bacteria)



### 2. Agar media:

- (Semi-solid, gel).
- the agar media is inside a plate.
- Growth is indicated by the formation of colonies.

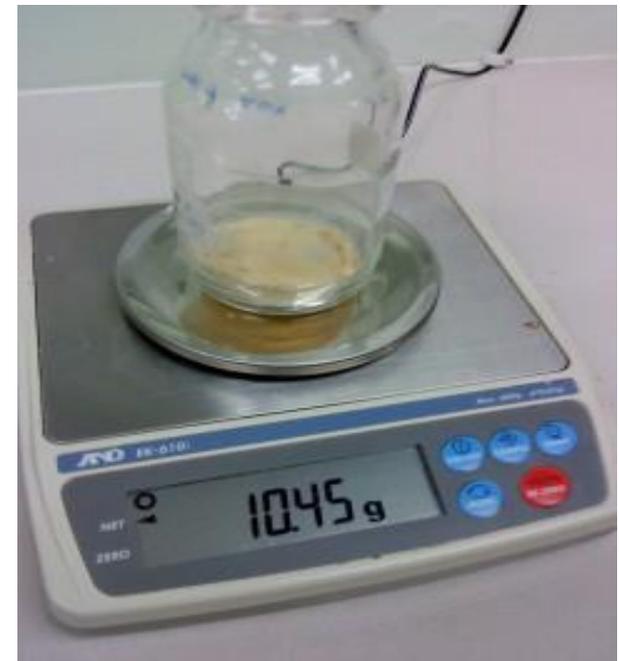


## Notes:

- Each colony is generated from a single bacterial cell.
- After we used the proper agar for our bacteria of interest, we put the agar media inside the incubator at a temperature of 37 C OR inside a water bag if we used liquid media which is also placed at a temperature of 37 C.
- The shape, size, color of the colonies give us an initial identification of the colonized bacteria.

The steps of agar preparation:

1. The agar is synthesized as powder and placed inside a bottle to increase its life and for better storage.
2. The recipe is written on each bottle (the recipes are different between different agar types) .
3. the recipe tells us the recommended amount for both agar powder and distilled water to produce the best texture for bacterial growth.



4. After mixing the components in a flask, we expose the mixture to a source of heat with rotation at the powder's melting point to ensure that all of the powder has dissolved in the distilled water(homogeneity), but the agar is still unsterilized.
5. the agar is sterilized by autoclave.
6. We then close the flasks and place them inside the autoclave for sterilization.



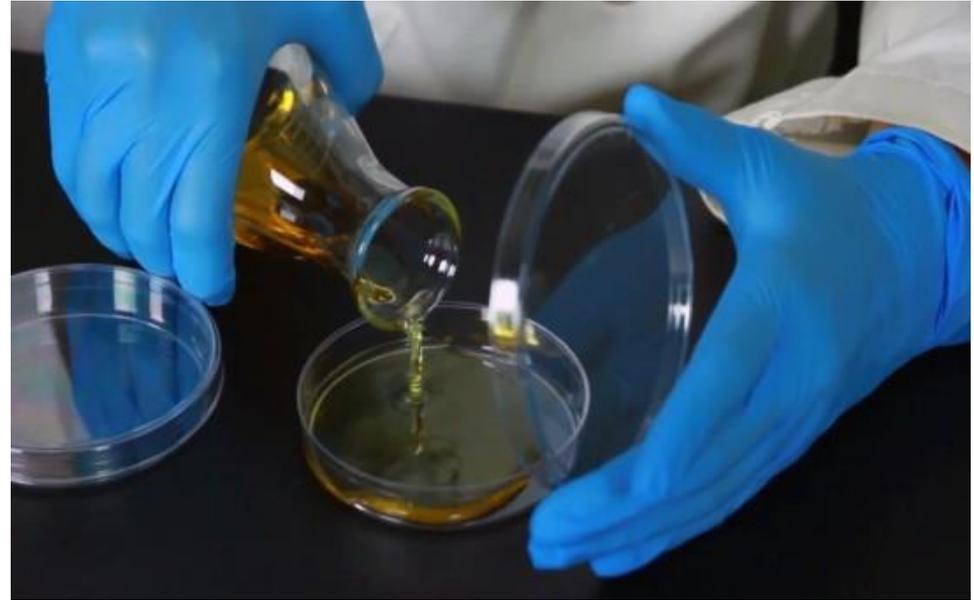


- **Autoclave condition:**
- Sterilization method
- 7. Important conditions are provided by autoclave that makes it more useful in sterilization:
  - High pressure 15 pound/inch.
  - High temperature 121 C.
  - Sufficient time (20-30) min.
-

Liquid media (broth)



Agar media



After autoclave is done:

We leave the mixture until its temperature become 55 C

If we made agar media, we put it inside the plate (petri dish) however if we made broth media, we put it inside the tubes.

the plate and the tubes are already sterilized from the factory.

- Extra step if we want to make blood agar media: After the sterilization is done.
- We add 7% sheep RBCs or human blood (for example: if we prepare 100L of agar media, we will add 7L sheep RBCs).
- Then we put the mixture inside the plates.
- chocolate agar media are boiled blood agar media, which cause denaturation of the RBCs



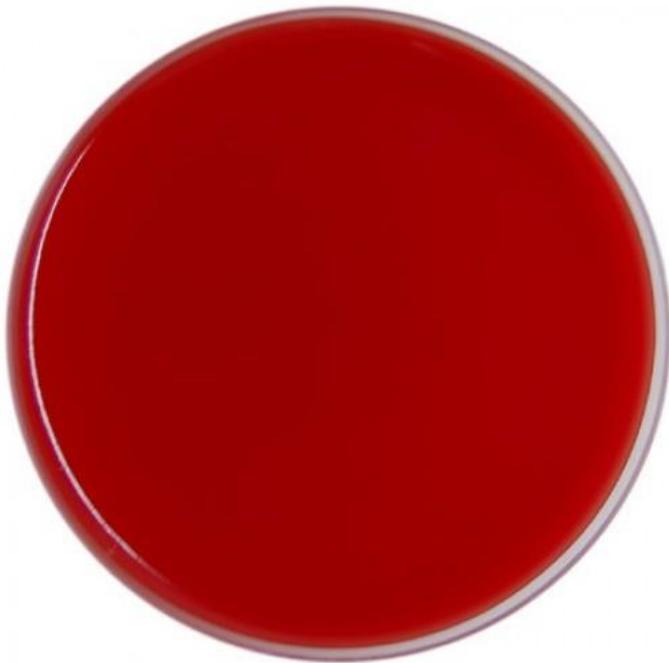
# Enrichment media

This type contain the nutrients required to support the growth of a wide variety of bacteria.

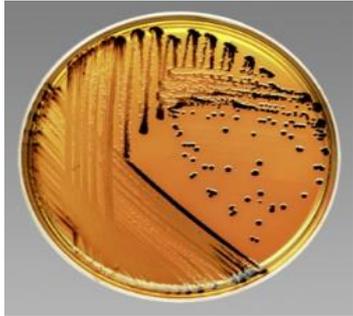
Like:

blood agar media

Mueller-Hinton agar



# Selective media



SS Agar Plate  
(Salmonella-Shigella Agar)

**selective media** is composed of specific ingredients to inhibit the growth of certain species of microbes in a mixed culture while allowing others to grow. **(Selection)**

Example of inhibitors that use in selective media:  
high Salt concentration, certain PH or antibiotic etc.....

Example of selective media:

1. Salmonella-shigella agar: which allow for both salmonella and shigella bacteria to growth.
2. MacConkey agar media: selective (allow) for only gram-negative bacteria to growth, uses in diagnosis of urinary tract infection(UTR).



Macconkey agar media

# Differential media

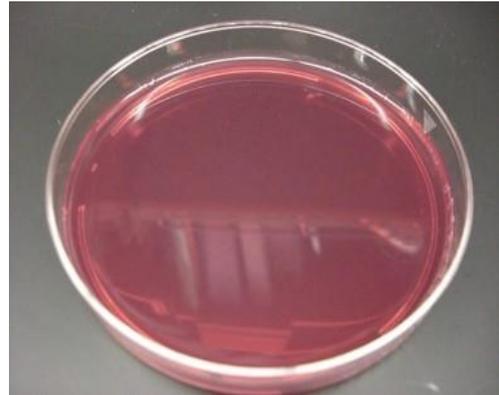
**Differential media:** that contain specific ingredients to allow one to distinguish selected species or categories of bacteria by visual observation (like change in color).

Examples:

- 1) MacConkey agar media: which can act as selective or differential media:
  - ✓ Selective: allow only for gram-negative (GN) bacteria to growth.
  - ✓ Differential: differentiate between lactose fermenter GN bacteria (PINK) and lactose non-fermenter GN bacteria (YELLOW)
- 2) Salmonella-shigella agar: also act as selective and differential media:
  - ✓ Selective: allow only for salmonella and shigella bacteria to growth.
  - ✓ Differential: differentiate between salmonella bacteria (black dotes) and shigella (colony transparent / no black dotes).



SS Agar Plate  
(Salmonella-Shigella Agar)



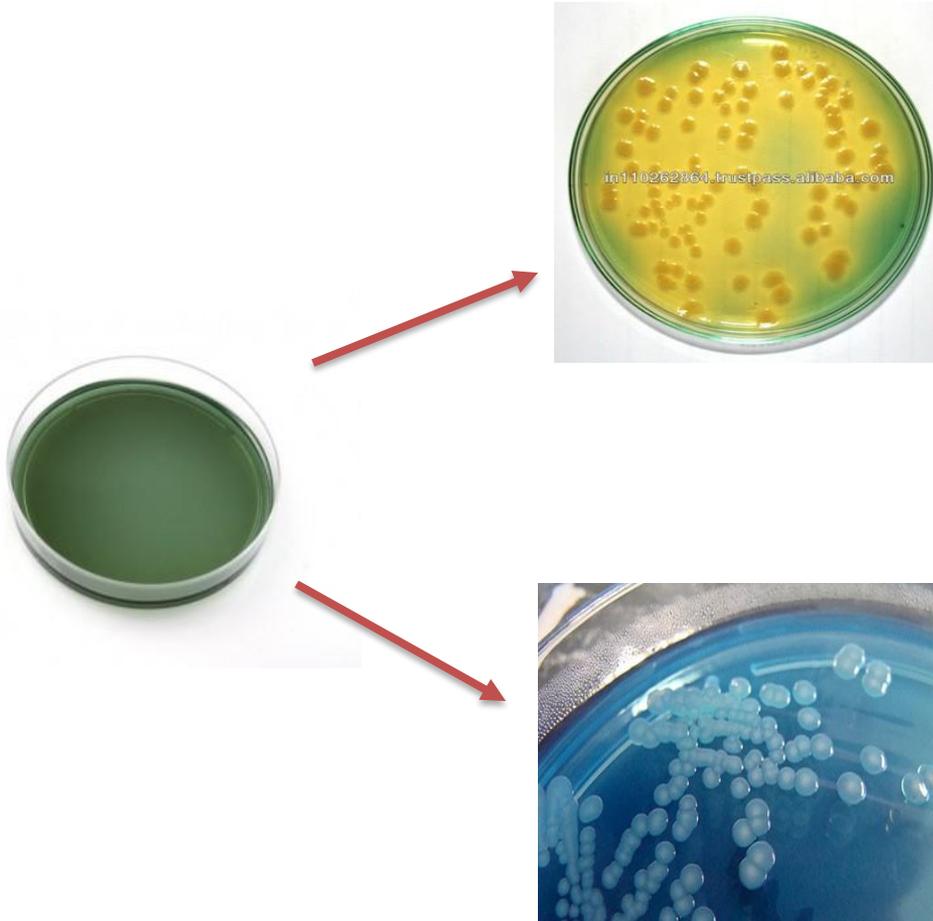
Macconkey agar media



Lactose fermenting colonies

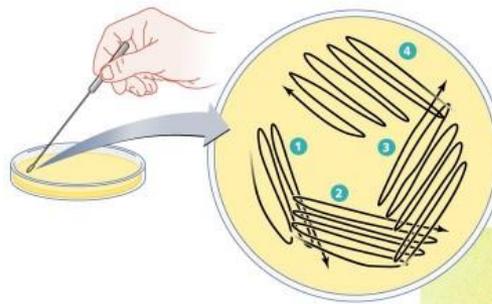
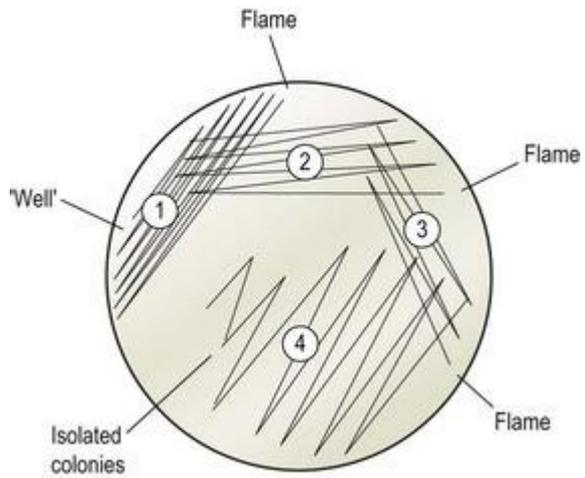
Non-lactose fermenting colonies

# Differential media



## **CLED agar (cystine lactose electrolyte deficient medium):**

- Also consider as differential media.
- Its original color is green.
- If the *Staphylococcus aureus* bacteria grow in CLED media, it will generate gold colony.
- However if the *Staphylococcus epidermidis* (*Staphylococcus Albus*) bacteria it will generate whit colon.

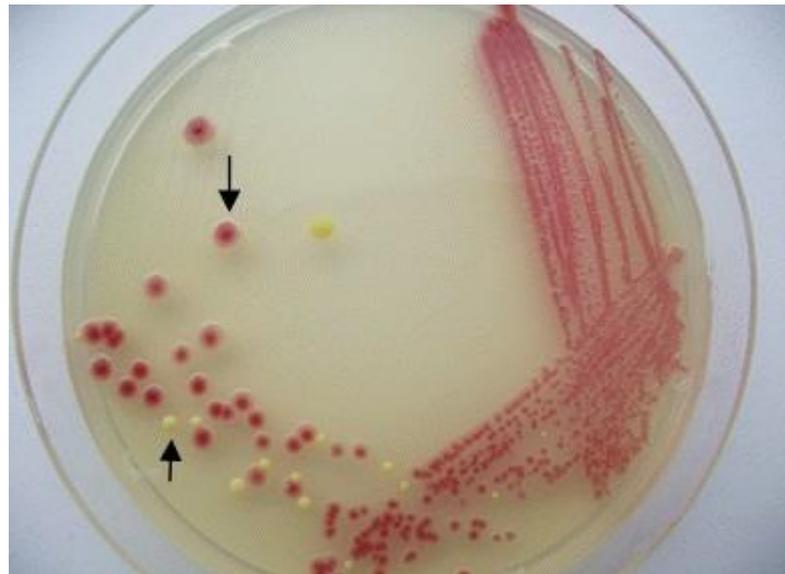
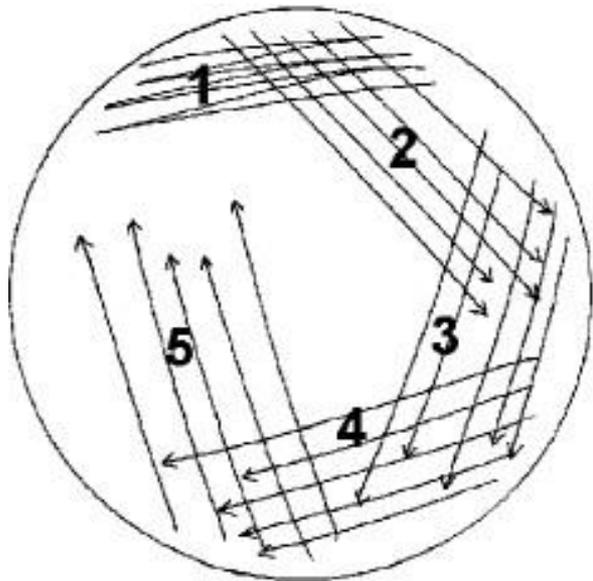


(a)



(b)

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## Bacterial culture (civilization):

- The purpose from bacterial culture is to produce single pure colonies on agar media, So if I have more than one type of bacteria in my sample, I can separate them by the morphology of that bacterial colony.
- Even that I have single type of bacteria in my sample, I need to have a pure (single) colony to do gram staining for identification and the sensitivity test for that type of bacteria.
- We do the bacterial culture by streak-plate method.  
the steps of streak-plate method in this link



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